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Developmental Stages of Egg Follicles in *Parnassius glacialis* BUTLER (Lepidoptera, Papilionidae)

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Abstract Oogenesis in *Parnassius glacialis* was studied by the use of light microscope. The structure of the ovary was described. The development of the egg follicles was divided into a series of consecutive stages ending with stage 12 on the basis of various morphological criteria. Structural changes of the cystocytes, oocytes, nurse cells and follicle cells were described.

Introduction

In Lepidoptera, a large number of investigations on the egg maturation, structure of the egg, fertilization and embryonic development have been made. The results obtained suggested that various developmental phenomena in the early embryogenesis might be controlled by the form, size and organization of the mature oocyte or egg. Therefore, understanding the formative process of the oocyte is important for making researches into the development of eggs and embryos. Although many reports have been published on the oogenesis of ditrysian Lepidoptera, *Bombyx mori* (MACHIDA, 1922, 1926, 1940, 1941; MIYA *et al.*, 1961 – 1970; KAWAGUCHI *et al.*, 1973 – 1986; YAMAUCHI *et al.*, 1981 – 1984; etc.), *Hyalophora cecropia* (TELFER *et al.*, 1960 – 1970; KING and AGGARWAL, 1965; STAY, 1965; etc.) and *Ephestia kühniella* (CRUICKSHANK, 1964 – 1972; CUMMINGS, 1972), there is no paper concerning the development of ovarian follicles in *Parnassius*.

This paper deals with the structures of the ovary and the characteristics of the developmental stages of *Parnassius glacialis* oogenesis, from the formation of cystocyte clusters to the completion of mature oocyte. It is the first report of subsequent researches on the ovarian follicles and early embryonic development.

Materials and Methods

Female adults of *Parnassius glacialis* BUTLER were captured in Gifu Prefecture in May. The living or fresh ovarioles which were taken out from the females were observed in Ringer's solution. When needed, nile blue or trypan blue were used for staining. For making sections, the ovarioles washed with phsiological salt solution were fixed with BOUIN's fluid, alcoholic BOUIN's fluid or CARNOY's fluid. The sections were cut 5 μm in thickness and stained with DELAFIELD's haematoxylin and eosin, HEIDENHEIN's iron-haematoxylin and eosin or toluidine blue.

Observations and Discussion

Structure of the ovary

The Ovary

The ovaries lying on either side of the alimentary canal are covered with the connective tissue which contains a network of numerous tracheae and muscles. Thay are surrounded by a great number of fat bodies in the young adult female. Fig.1 shows a pair of ovaries containing developing egg follicles and the oviducts possessing chorionated oocytes. Each ovary is composed of four egg tubes which are the polytrophic meroistic ovarioles, roughly 9 mm long from the tip of the terminal filament to the pedicel. The pedicels converge and open posteriorly into the lateral oviduct, approximately 7 mm long. The entire ovariole is sheathed in two transparent membranes; an outer epithelial sheath and an inner acellular elastic layer, that is, the tunica propia. The two lateral oviducts connecting with the pedicels fuse in the mid-line and posteriorly join the common oviduct, about 3 mm long.

A young adult female possesses only a few mature oocytes in the posteriormost region of the vitellarium or the lateral oviducts and lays 80 – 100 eggs during a week. Therefore, the majority of fully formed oocytes which will be laid must be produced

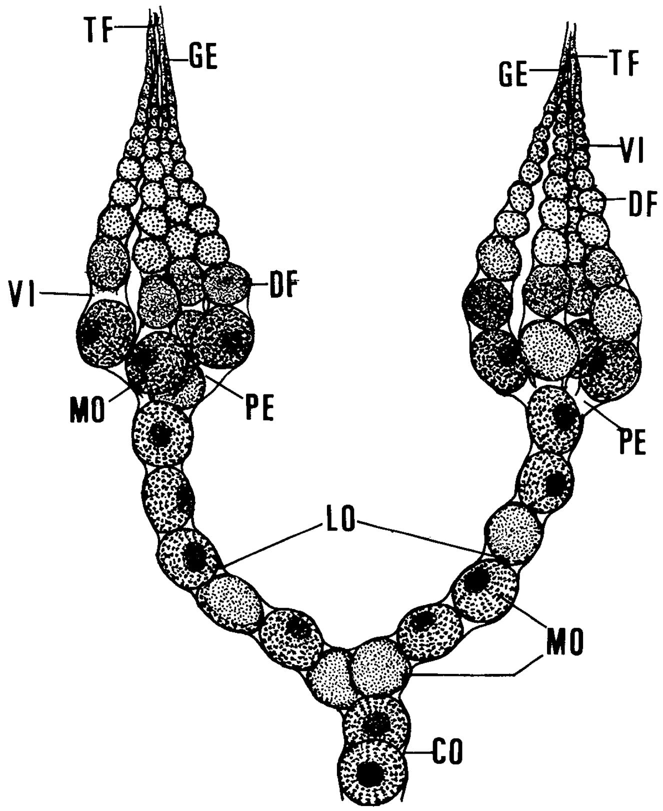


Fig. 1. Diagram of a pair of ovarioles and oviducts of an adult female. CO : common oviduct ; DF : developing follicle ; GE : germarium ; LO : lateral oviduct ; MO : mature oocyte ; PE : pedicel ; TF : terminal filament ; VI : vitellarium.

during the adult stage.

The ovarioles

Each of four ovarioles constituting an ovary is a separate, elongate tube which contains a large number of oogonia, cystocytes and developing egg follicles in a thin ovariolar wall. The vitellarium of each ovariole of the young female contains an average of 25 maturing egg follicles. Although MAHOWALD (1972) described that the polytrophic ovariole could be divided into five regions on the basis of general morphology, I divided each ovariole of *Parnassius* into the following four zones or regions antero-posteriorly.

1. The terminal filament which forms a slender apical prolongation of the ovariole. This is composed of the thread-like cells and fused distally with other three filaments to form a suspensory ligament attached to the dorsal body wall.

2. The germarium which contains the oogonia, cystoblasts, cystocytes and prefollicular cells. This is the zone from the basal part of terminal filament to 200–300 μm long posteriorly, following the vitellarium and may be divided into two i.e., anterior and posterior regions. The former contains many oogonia, cystoblasts and somatic cells which are distributed freely, whereas the latter contains many cystocyte clusters and prefollicular cells.

3. The vitellarium which contains linear series of the egg follicles. The anterior portion of the vitellarium contains small oocyte-nurse cell complexes lying in single file. The developing egg follicles are composed of one oocyte and a solid mass of 7 nurse cells surrounded by a single layer of the follicular epithelium derived from the mesodermal cells. They become successively larger and more mature towards the posterior end of the vitellarium, so that the oldest oocytes or the mature ovarian eggs are situated nearest the union with the lateral oviduct.

4. The pedicel which is the ovariole duct uniting the vitellarium with the lateral oviduct. The four pedicels unite at the distal end to form the calyx which opens into the lateral oviduct.

Oogenesis

In *Parnassius glacialis*, since each ovariole of an adult female contains the oogonia, cystoblasts, cystocyte clusters, developing egg follicles and mature oocytes with the chorion, the entire process of oogenesis may be investigated by observing developing cells situated in the germarium and vitellarium.

For understanding of oogenesis in the lepidopterous insects, the gross period of development of the egg follicles has been divided into many stages by several investigators. CRUICKSHANK (1971) and TORRES (1981) divided oogenesis into 9 stages in *Ephestia*, and KING and AGGARWAL (1965) into 12 stages in *Hyalophora*. In *Bombyx*, OZAWA (1959) divided follicular development into 7 stages, MIYA and KURIHARA (1966) and MIYA et al. (1970a) into 6 stages, YAMAUCHI et al. (1981) divided the process of oogenesis excluing choriogenesis into 10 stages. More recently, YAMAUCHI and

YOSHITAKE (1984) divided the entire developmental process of *Bombyx* ovarian follicles into a series of consecutive stages ending with 12 stages, the mature egg. In the present study, for making a detailed comparison between the oogenesis of *Parnassius* and those of other lepidopteran insects, I divided the process of oogenesis, from the cystoblast formation to the completion of mature oocyte, into consecutive stage 12 in conformity to division by KING and AGGARWAL (1965), YAMAUCHI and YOSHITAKE (1984). The morphological criteria used for the stage assignments were adopted from those proposed by YAMAUCHI *et al.* (1981) for *Bombyx* oogenesis. They are as follows: 1) size, shape and arrangement of oocyte-nurse cell complex in the ovariole, 2) shape and position of nuclei of the oocyte and nurse cells, and morphology of chromatin and nucleoli, 3) size ratio in egg and nurse chambers, 4) increase in number or migration and intercellular spaces of follicular cells, 5) formation of vitelline membrane and chorion. Table 1 shows the staging criteria for *P. glacialis* oogenesis.

Formation and development of egg follicles during previtellogenesis

Stage 1 (Fig.2 - S1A, 1B)

The cells in the first stage of oogenesis are found in the germarium. A large number of oogonia, cystoblasts and somatic cells derived from the mesodermal cells lie irregularly in the anterior region of germarium. The cystoblasts are oval in shape, 10 – 15 μm in diameter and have a large nucleus, while the somatic cells are of irregular form and much smaller than the oogonia and cystoblasts.

In the adjoining part, the cystocyte clusters consisting of 8 cystocytes produced by three successive divisions of the cystoblast are seen. The number of the cystocytes derived from a cystoblast is determined by the 2^n rule, and that of the nurse cells is expressed by $2^n - 1$ as described by TELFER (1975). The cystocytes, about 20 μm in diameter, are interconnected by 7 cytoplasmic canals i.e., the intercellular bridges, as observed in various ditrysian cystocyte clusters (KNABEN, 1934; HIRSCHLER, 1942; KING and AGGARWAL, 1965; MIYA *et al.*, 1970b; CAVE and SIXBEY, 1976; CAVE, 1978; MANDELBAUM, 1980; YAMAUCHI *et al.*, 1981; YAMAUCHI and YOSHITAKE, 1984; GRIFFITH and LAI-FOOK, 1986). The cystocyte nuclei, spherical in shape, possess one or two nucleoli and several chromatin masses or short chromosomes. In these earliest cystocytes morphological difference among them is not yet recognizable. The prefollicular cells differentiated from the somatic cells arrange themselves along the periphery of the germarium, that is, the inside of the tunica propia. Some of them intervene among the cystocyte clusters.

Stage 2 (Fig.2-S2)

All the cystocytes are the same size and about 25 μm in diameter. One cystocyte moves backwards and now lies at the posteriormost part of the cell cluster to develop into the oocyte, while other 7 cystocytes differentiate into the nurse cells. During this course the cystocyte clusters or the oocyte-nurse cell complexes lying in the anterior

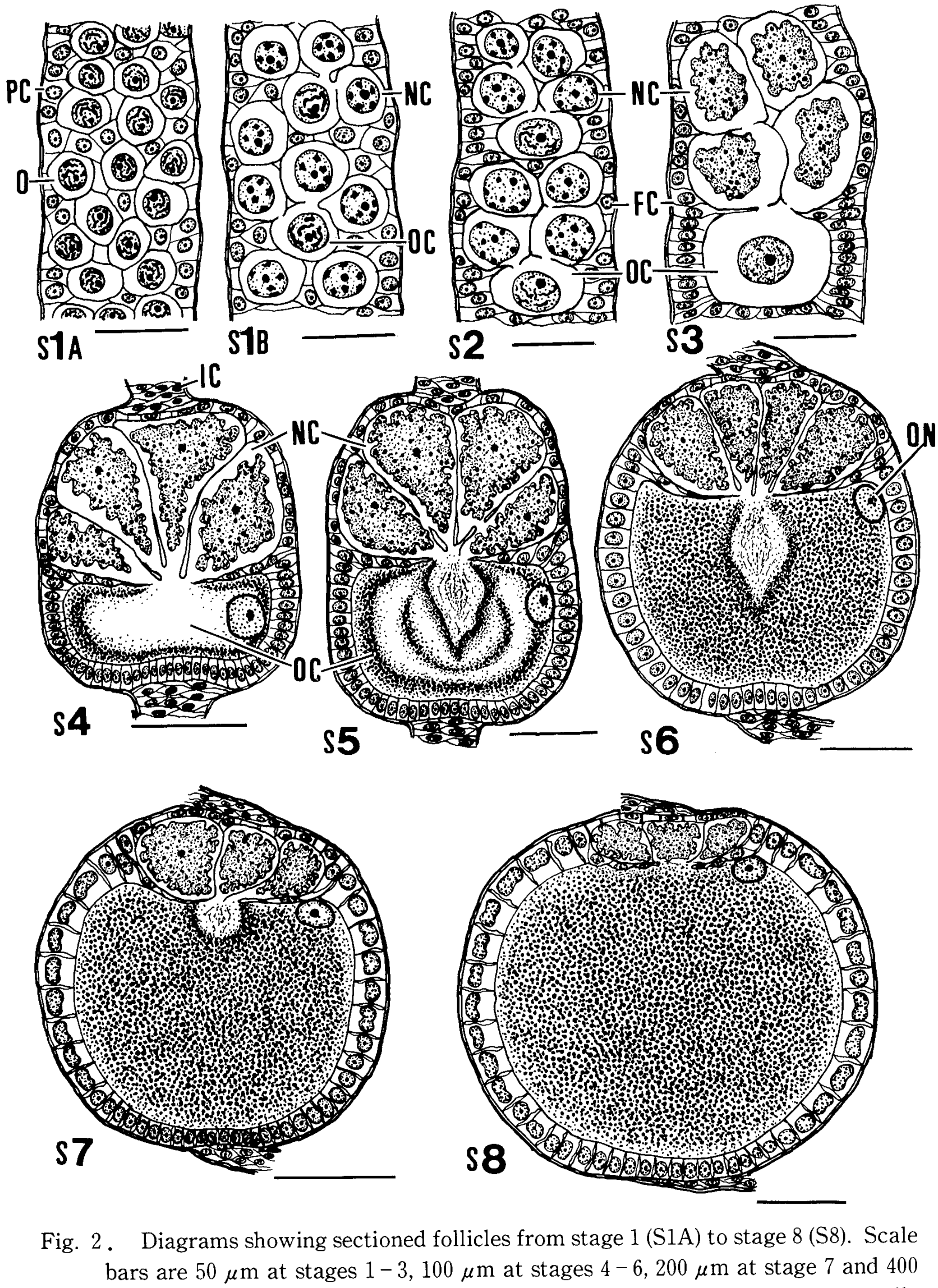


Fig. 2. Diagrams showing sectioned follicles from stage 1 (S1A) to stage 8 (S8). Scale bars are 50 μm at stages 1–3, 100 μm at stages 4–6, 200 μm at stage 7 and 400 μm at stage 8. FC : follicle cell ; IC : interfollicular connective ; NC : nurse cell ; O : oogonium ; OC : oocyte ; ON : oocyte nucleus.

region of the vitellarium are disposed in single file owing to the increasing in the cystocyte volume and begin to be enclosed by the follicle cells.

As development progresses, the oocyte nucleus grows into a germinal vesicle, losing the affinity for dyes. On the other hand, the nurse cell nuclei, containing a lot of dispersed chromatin granules and several condensed masses of chromatin, increase

in their volume faster and lose their smooth outline gradually. Consequently, the oocyte can be clearly distinguished from the nurse cells morphologically. As the oocyte-nurse cell complexes are progressively covered with the follicle cells, yolk substance is accumulated in the cytoplasm. In the fresh materials immersed in physiological salt solution, a great number of lipid droplets are found in the cytoplasm of the oocyte, nurse cells and follicle cells, as observed in *Bombyx* follicles (MACHIDA, 1940, 1941; AGGARWAL, 1962; MIYA and KURIHARA, 1966; MIYA *et al.*, 1969 ; YAMAUCHI *et al.*, 1981; YAMAUCHI and YOSHITAKE, 1984). In *Hyalophora* follicles at this stage, glycogen granules flowing into the oocyte from the nurse cells through the cytoplasmic bridges were observed (KING and AGGARWAL, 1965).

Stage 3 (Fig.2-S3)

The oocyte and 7 nurse cells increase in their volume at similar rates, but the nurse cell nuclei grow more rapidly than the oocyte nucleus. As the oocyte-nurse cell complex grows, the nurse cell nuclei become somewhat amoeboid, and many chromatin masses appear in the nucleoplasm, while the oocyte nucleus located in the central position of the ooplasm gradually lose the basophilia, though a prominent, large nucleolus maintains its appearance. In a short time the oocyte-nurse cell complexes are almost completely enclosed by the follicle cells and are separated from adjacent complexes.

With the lapse of time the follicle cells over the oocyte, arranging themselves in a single cell layer consisting of about 40 cells in a sagittal section, increase in number and in thickness to form columnar cells, whereas those over the combined nurse cells remain in original form, being almost cuboidal. Several follicle cells migrate centripetally between the oocyte and nurse cells. As the oocyte and the combined nurse cells are enclosed completely by follicular epithelium, except the intercellular cytoplasmic bridges, i.e., the nutritive pore, the egg chamber and the nurse chamber are formed. The follicle cells migrating into or intervening between the oocyte-nurse cell complexes become flattened and are arranged vertically for the antero-posterior axis of the ovarioles. These cells contribute to the formation of interfollicular tissue connecting with each complexes. Towards the end of this stage the follicle cells over the combined nurse cells become gradually flattened, whereas those over the oocyte become increasingly columnar.

Development of egg follicles during vitellogenesis

Stage 4 (Fig. 2-S4)

At the onset of this stage the oocyte is hemispherical in shape and its volume is one-half of that of combined nurse cells, that is, one-third of the volume of the oocyte-nurse cell complex. A solid mass of the nurse cells is also hemisphere. The oocyte grows more rapidly than the nurse cells at this stage. The nurse cell nuclei are amoeboid in shape and contain a large number of chromatin masses and granules throughout the nucleoplasm, whereas the oocyte nucleus is almost spherical, about 30 μm in diameter, light-stained and possesses one large nucleolus. The interfollicular

tissue connecting neighbouring oocyte-nurse cell complexes form an interfollicular stalk, that is, the interfollicular connective.

Towards the end of this stage the oocyte grows large rapidly into an enomous cell owing to the supply of yolk substances or cytoplasmic components from the nurse cells through the nutritive pore. With the increase in volume of the ooplasm by the accumulation of yolk materials, the oocyte nucleus shifts from a central position to the dorsal side of the ooplasm. The nucleolus of the oocyte nucleus divides several pieces, as observed in *Bombyx* (MACHIDA, 1940, 1941; YAMAUCHI *et al.*, 1981; YAMAUCHI and YOSHITAKE, 1984). At the end of this stage proteid yolk spheres begin to accumulate in the periphery of the ooplasm.

Stage 5 (Fig. 2-S5)

At the onset of this stage the oocyte volume is almost equal to that of the combined nurse cells, that is, one-half of the egg follicular volume. The oocyte nucleus lying at the lateral side of the ooplasm is elliptical, about 65 by 40 μm in size, in contact with the cell membrane and possesses a group of various sized chromatin fragments originated from the nucleolus. Each nurse cell nucleus possesses a similar chromatin mass and contains a great number of chromatin bodies and granules. The streaming of active supply of nutrients from the nurse cells into the oocyte and numerous, very tiny granules distributed around the stream are remarkably visible in a section. After the oocyte volume became twice as large as that of the combined nurse cells, the nurse cells rearrange themselves in a row. With the growth of oocyte, the follicle cells over the lateral sides of the oocyte grow increasingly, and the cells lying between the oocyte and nurse cells become squamous. The inner and outer cytoplasm of the follicle cells surrounding the oocyte is deeply stained with basic dyes.

Towards the end of this stage the narrow spaces appear partially between the lateral sides of the follicle cells and between the inner surface of the follicular epithelium and the oocyte. The intercellular spaces are also formed between the follicular epithelium and nurse cells.

Stage 6 (Fig. 2-S6)

At the beginning of this stage the oocyte volume accounts for three-quarters of the egg follicular volume, about 390 by 360 μm in size. The nurse chamber become elliptical in shape. The anterior side of the oocyte is flat, whereas the posterior side is convex. The oocyte nucleus, about 70 by 40 μm in size, lies in the antero-lateral position of the ooplasm. As the oocyte grows large, the egg follicle rotates and its antero-posterior axis becomes mutually inclined on either side at the angle of about 45 degrees to the ovariolar wall. At the time when the oocyte reaches four-fifths of the egg follicular volume, about 450 μm in diameter, basophilic material accumulated in the inner part of each follicle cell cytoplasm is released into the intercellular spaces between the follicular epithelium and the oocyte (Fig.4). Then it flows into the oocyte to form a large number of yolk granules of varying size in the periphery of the ooplasm (Fig. 5). Joining with another to form the large spheres, the yolk granules move centripetally. The investigations on protein synthesis and accumulation in the ovaries of various lepidopteran insects have been made by the various techniques; electron

microscopic, immunological and autoradiographic studies. The results obtained show that the proteid yolk spheres are formed by two different ways: one of which is that the protein synthesized in the nurse cells and the follicle cells is transfered and accumulated in the oocyte (OTSUKI, 1965, in *Bombyx*; MIYA *et al.*, 1969, 1970a, in *Bombyx*; ANDERSON and TELFER, 1969, in *Hyalophora*; CRUICKSHANK, 1971, in *Anagasta*; KAWAGUCHI and FUJII, 1984, in *Bombyx)*, and the other is that the protein produced in the fatty tissue is secreted into haemolymph, then it accumulates in the oocyte through the intercellular spaces of the ovary (TELFER, 1960, 1961, in *Hyalophora*; TELFER and MELIUS, 1963, in *Hyalophora*; STAY, 1965, in *Hyalophora*; KAWAGUCHI and DOIRA, 1974, in *Bombyx*; FUJII and KAWAGUCHI, 1983, in *Bombyx*). The proteid yolk spheres of *Parnassius* oocyte seem to be formed by both the two ways above-mentioned.

Immediately after the formation of yolk spheres in the periphery of the ooplasm, a lot of granules dark-stained with iron-haematoxylin make their appearance around the core of the oocyte, and then they fuse each other to produce the yolk spheres being a close resemblance to those derived from the secretion of the follicle cells. Consequently, the oocyte possesses two kinds of proteid yolk spheres: one of which is synthesized by the oocyte itself, and the other is supplied by the follicle cells and haemolymph.

Stage 7 (Fig. 2-S7)

The egg follicle becomes almost spherical in shape and reaches the size of about 700 μm in diameter. The oocyte volume accounts for six-sevenths or more of the egg follicular volume. The nurse cell nuclei reach their maximum size. After the follicle cells over the lateral sides of the oocyte grow and become square in shape, their nuclei become constricted in the middle to divide into two amitotically (Fig. 6). On the other hand the follicle cells over the posterior region remain small in size and columnar in shape. In the latter half of this stage the follicle cells increase in number from about 40 to 45 in the sagittal section through the egg chamber, because the amitotic nuclear fission leads to division of the cytoplasm. Consequently the whole surface of the oocyte is covered with 600 follicle cells, roughly estimated. The rapid growth of oocyte by accumulation of nutrient material seems to result in the increase in size of the interfollicular cell spaces. The nurse cells are gradually compressed by the growing oocyte to form an anterior disc.

Stage 8 (Fig. 2-S8)

The oocyte is spherical in shape and about 800 μm in diameter. The cytoplasmic bridges between the oocyte and nurse cells are not yet closed, but the streaming of nutrients into the oocyte is hardly visible. Consequently, the continuous increase in oocyte volume seems to be due to the supply of protein yolk from the follicle cells and the incorporation of haemolymph protein through the intercellular spaces. Many follicle cells over the antero-lateral and lateral zones of the oocyte continue to divide amitotically to increase in their number, while those over the posterior zone grow large rapidly, but they do not divide. Towards the end of this stage the nurse cells and their nuclei shrink gradually and terminate nutrient supply into the oocyte. The follicle cells proliferated amitotically begin to migrate centripetally between the oocyte and the

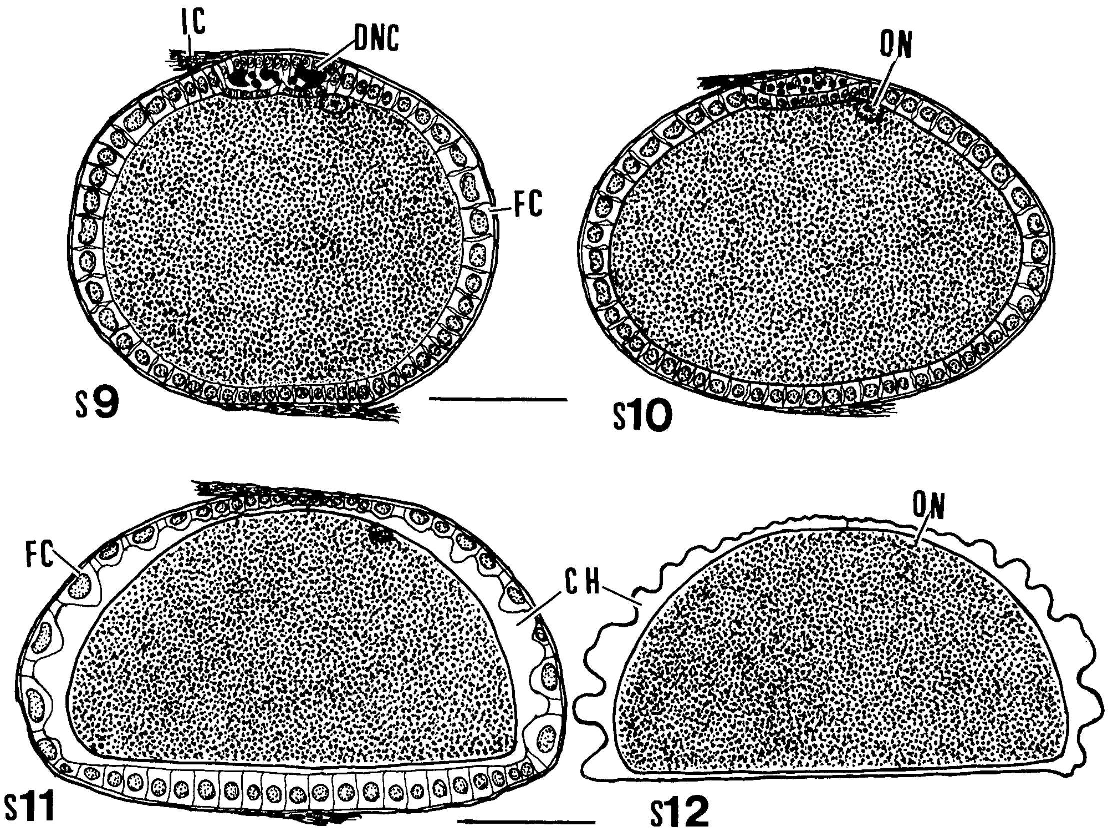


Fig. 3. Diagrams showing sectioned follicles from stage 9 (S9) to stage 12 (S12). Scale bars are 400 μm. CH : chorion; DNC : degenerating nurse cell; FC : follicle cell; IC : interfollicular connective; ON : oocyte nucleus.

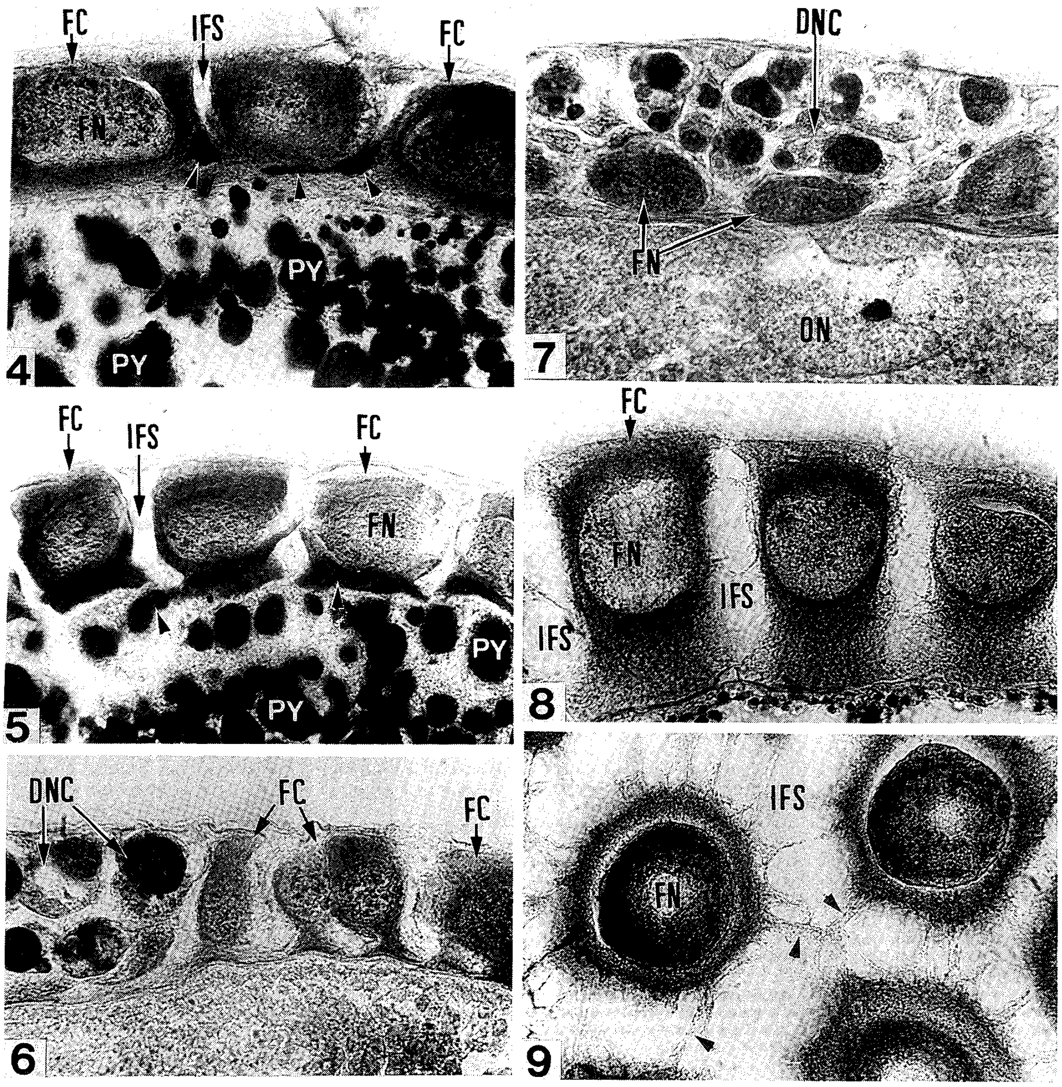
mass of degenerating nurse cells. The squamous follicle cells, lying near the center of the septum which separates the oocyte from the nurse cells, produce small cells (micropylar-forming cells) destined to form the micropylar apparatus.

Stage 9 (Fig. 2-S9)

The oocyte is about 1.1 mm in width, 0.9 mm in height and enclosed by 50 or more follicle cells in a sagittal section. The antero-posterior axis, namely short axis of the egg follicle is perpendicular to the surface of an egg tube. The oocyte nucleus is about 85 μm in size and possesses a large nucleolus. Nuclear and cytoplasmic volume of the follicle cells continue to increase during this stage. The nurse cell nuclei break down and begin to produce the chromatin masses (Fig. 7). Many vacuoles of varying size appear throughout the nurse cell cytoplasm. Since the bridges between oocyte and nurse cells are completely closed by the central migration of the follicle cells, the oocyte is sealed off from the mass of degenerating nurse cells by a single cell-layer. The micropyle-forming cells are situated as a group of small cells in the central position of the septum separating the oocyte from the nurse cells.

Stage 10 (Fig. 3-S10)

The oocyte reaches 1.0 by 1.3 mm in size and is covered with about 60 follicle cells in a sagittal section. Its posterior region becomes somewhat flattened. The follicle



Figs. 4 − 9. Stages of egg follicles of *Parnassius glacialis*. 4, 5. Cross sections through the lateral regions of egg follicles at stage 9. Arrowheads show condensed basophilic material, protein. 6, 7. Sagittal sections through the anterior region of egg follicle in vitellogenesis (6: stage 7; 7: stage 9). 8, 9. Sections through follicle cells at stage 10 (8: logitudinal section; 9: cross section). Arrowheads show cytoplasmic filaments which bridge intercellular spaces. DNC : degenerating nurse cell; FC : follicle cell; FN: follicle cell nucleus; IFS: interfollicular cell space; ON: oocyte nucleus; PY: proteid yolk sphere.

cells are maximally separated from each other (Fig. 8), but they keep in contact with neighboring cells by numerous cytoplasmic filaments which bridge the intercellular spaces (Fig. 9). The follicle cells are more deeply stained with haematoxyline, because the previtelline bodies or chorionic material are actively synthesized in cytoplasm. The amitotic figures of the follicle cells are hardly observed at this time. The chromatin masses in degenerating nurse cells are caught phagocytically by the surrounding follicle cells. The formation of proteid yolk spheres in the oocyte terminates.

At the time when the yolk is fully accumulated, the inner parts of the oocyte

contain a myriad of large yolk spheres of varying size, whereas the outer or peripheral parts contain small yolk granules, including the tertiary protein granules which are distinguishable from other ones by difference of affinity for dyes, as observed in *Anagasta* (CRUICKSHANK, 1972a, b), in *Bombyx* (TAKESUE *et al*., 1976, 1980; YAMAUCHI and YOSHITAKE, 1984), in *Hyalophora* (RUBENSTEIN, 1979) and in *Amata* (TANAKA, 1985). Towards the end of this stage the vitelline membrane is formed in the intercellular spaces between the follicle cells and oocyte. Regarding the origin of vitelline membrane there are various different opinions among the investigators. CUMMINGS (1972) described for *Ephestia* that the previtelline bodies synthesized in the follicle cells were secreted into the spaces between the oocyte and follicle cells, where they fused to form a continuous membrane. CRUICKSHANK (1971, 1972a) reported in *Anagasta* that both oocyte and follicle cells synthesized protein used in the construction of the vitelline membrane. KIM *et al*.(1983) obtained the similar result in *Pieris*. MACHIDA (1940, 1941) and MATSUZAKI (1968) observed in *Bombyx* that the fully grown oocyte produced granules which formed the vitelline membrane. AKUTSU and YOSHITAKE (1977), however, described for *Bombyx* that the vitelline membrane seemed to be formed basically by zona pellucida with granules, as material, which were secreted from the follicle cells. Recently, GRIFFITH and LAI-FOOK (1986) reported in *Calpodes* that the vitelline membrane appeared to be secreted largely by the oocyte first, and then the material from the follicle cells was probably added to it.

Development of egg follicles during choriogenesis

Stage 11 (Fig. 3-S11)

The egg follicle is about 1.4 mm in width, 1.0 mm in height and echinus in shape; the anterior side is convex and the posterior one flat. The cortical layer of oocyte i. e., the periplasm is organized rapidly at the beginning of this stage. The degenerating nurse chamber containing many chromatin masses remains on the follicular epithelium enclosing the dorsal side of the oocyte. The follicle cells rejoin apically but are still follicular separated greatly in their lateral parts.

In the early part of this stage the follicle cells begin to secrete chorionic material to form the eggshell. At the early period of choriogenesis the chorionic layer is uniformly formed, because all follicle cells secrete chorionic material at approximately the same rates over the vitelline membrane. After the chorion reaches about 20 μm in thickness, the follicle cells intensely deposit secretions within the intercellular spaces. The secretory activity differs among the follicle cells enclosing the different regions of the oocyte, especially the cells over the lateral sides of the oocyte show a phase of intense secretory activity of chorionic material to form the chorionic layers which are composed of lamellae running in different directions. As the follicle cells produce a series of secretions, they decrease in cytoplasmic volume to become cone-shaped. During this stage, large pits are formed on the chorion over the lateral sides of the oocyte, opposite to each follicle cell. In the last stage of choriogenesis, the columnar follicle cells over the posterior flattened surface of the chorion rapidly

become greatly swollen and lose the affinity for basic dyes. At the end of this stage the oocyte nucleus embedded in the cytoplasmic island enters the first meiotic metaphase.

Stage 12 (Fig. 3-S12)

The chorion is completed. The chorionated oocyte, lying at the posterior end of the vitellarium, is about 1.4 by 0.8 mm in size. The mature oocytes or eggs slough off a layer of degenerating follicular epithelium and is liberated into the lateral oviduct.

Table 1. Stages of *Parnassius* oogenesis.

Stage	Diagnostic characteristic of developmental stage
1	Eight cystocytes are formed by three successive divisions of cystoblast. Cystocyte clusters begin to arrange in a row.
2	Oocyte-nurse cell complexes are arranged in single file and enclosed by follicle cells. Oocyte moves backwards to lie at posteriormost portion of cystocyte cluster.
3	Nurse cell nuclei become amoeboid and chromatin disperses nucleoplasm. Centripetal migration of follicle cells takes place at oocyte-nurese cell interface.
4	Oocyte accounts for one-third of egg follicular volume. Proteid yolk spheres begin to accumulate in periphery of ooplasm.
5	Oocyte accounts for one-half of egg follicular volume. Intercellular spaces begin to expand between of follicle cells over oocyte.
6	Oocyte accounts for four-fifths of egg follicular volume. Oocyte nucleus lies in antero-lateral position of ooplasm.
7	Oocyte accounts for six-sevenths or more of egg follicular volume. Nurse cell nuclei reach maximum volume. Follicle cells surrounding oocyte divide amitotically.
8	Oocyte is almost spherical. Nurse cells become anterior disc and begin to degenerate. Follicle cells migrate centripetally between oocyte and nurse cells.
9	Oocyte is completely sealed off by a layer of follicular epithelium. Nurse cell nuclei break down and begin to produce chromatin masses.
10	Posterior region of oocyte becomes somewhat flattened. Follicle cells are maximally separated from each other. Formation of proteid yolk spheres terminates. Vitelline membrane is completed.
11	Chorion formation commences. Oocyte nucleus enters first meiotic metaphase. Follicle cells begin to degenerate.
12	Chorion is completed. Mature egg sloughs off a layer of follicular epithelium and is liberated into lateral oviduct.

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摘　要

ウスバシロチョウにおける卵胞の発達段階（田中正弘）

ウスバシロチョウ *Parnassius glacialis* の卵巣の構造と卵形成の過程を光学顕微鏡で観察した.

（卵巣）

卵巣は結合組織と脂肪体でおおわれ，4つの交互栄養型卵巣小管から構成されている. 各卵巣小管はおよそ9mmで，前端より端系（terminal filament），生殖巣（germarium），卵黄巣（vitellarium），卵管柄（pedicel）に分けられる. 生殖巣は卵原細胞（oogonium），嚢胞胚（cystoblast），嚢胞細胞（cystocyte），前濾胞細胞（prefollicle cell）を有し，卵黄巣から卵管柄までの間に平均して25の卵胞（egg follicle）が存在し，それらは発達の段階順に一列に配列している.

（卵形成）

卵胞の発達過程は，嚢胞細胞，卵母細胞（oocyte），栄養細胞（nurse cell），濾胞細胞（follicle cell）の形態的特徴にもとづいて12のステージに分けられる. 各ステージにおける卵胞の発達状態は次のようである.

ステージ1；嚢胞胚の連続する3回の分裂により，8個の嚢胞細胞が形成され，それらは相互に細胞質の橋で連絡している.

ステージ2；嚢胞細胞の1つが後方へ移動して卵母細胞になり，他の7つの嚢胞細胞は栄養細胞になる. 卵母細胞—栄養細胞複合体（oocyte-nurse cell complex）は1列に配列し，それらは次第に濾胞細胞でおおわれる.

ステージ3；栄養細胞核は急速に成長し，多数のクロマチン粒を含むアメーバ状となる. 卵母細胞—栄養細胞複合体は濾胞細胞で完全に包まれ，隣接する複合体から分離される. 間もなく，卵母細胞を包む濾胞細胞は円柱状となり，一層の濾胞上皮を形成する. 卵母細胞と栄養細胞群との間に濾胞細胞が侵入し，卵胞は卵室（egg chamber）と栄養細胞室（nurse chamber）とに分けられる.

ステージ4；卵母細胞は半球状となり，その体積は卵胞全体の1/3になる. 栄養細胞から卵母細胞への栄養物質と細胞構成要素の流入が顕著になる. 卵母細胞内での栄養物質の蓄積に伴い，卵母細胞核は側方へ押しやられ，仁は崩壊して多数の小片になる.

ステージ5；卵母細胞は卵胞全体の1/2の大きさになる. 卵胞全体を包む濾胞細胞間に狭い空隙が生じ，卵母細胞の表層域にタンパク性卵黄小球が形成される.

ステージ6；卵母細胞は卵胞全体の3/4を占める. 卵胞が回転し，その前—後軸は卵管軸に対して45度ほど傾く. 卵母細胞の内部に於いてもタンパク性卵黄小球の形成が進む.

ステージ7；卵胞はほぼ球形となり，卵母細胞は卵胞全体の6/7に達する. 卵母細胞の側方を包む濾胞細胞は大きさを増して立方形になり，その後無糸分裂によって増殖する.

ステージ8；直径0.8mmに達した卵母細胞は徐々に扁平楕円体へと変形し始める. 栄養細胞群は細胞の前部で圧縮され，退化し始める. 栄養細胞室と卵室との間に無糸分裂で増殖した濾胞細胞が侵入する.

ステージ9；卵母細胞は0.9×1.1mmの大きさに達し，前—後軸は卵管軸と直交する. 栄養細胞核が崩壊し，多数のクロマチン粒を形成する. 卵室は濾胞細胞層によって栄養細胞室から完全に分離される.

ステージ10；卵母細胞は1.0×1.3mmの大きさに達し，その後極部が扁平となる. 濾胞細胞間の空隙が著しく拡大するが，細胞質は細い橋で連絡している. 栄養細胞核から生じた大小のクロマチン粒は濾胞細胞に取り込まれる. 卵母細胞の成長が終了し，卵黄膜が形成される.

ステージ11；卵室は1.0×1.4mmの「まんじゅう」形となり，卵母細胞の表層構造が完成する. 濾胞細胞から分泌される卵殻形成物質が卵黄膜上に蓄積し，卵殻の形成が進む. 卵母細胞核が第一成熟分裂の中期の状態に入る.

ステージ12；卵殻が完成し，0.8×1.4mmに達した卵母細胞は濾胞上皮から離脱し，側輸卵管内へ移行する.